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<b>(21) International Application Number:</b> PCT/AU92/00423 <b>(22) International Filing Date:</b> 10 August 1992 (10.08.92) <b>(30) Priority data:</b> PK 7725 13 August 1991 (13.08.91) AU <b>(71) Applicants (for all designated States except US):</b> BIOTECH AUSTRALIA PTY. LIMITED [AU/AU]; 28 Barcoo Street, Roseville, NSW 2069 (AU). ST VINCENT'S HOSPITAL SYDNEY LIMITED [AU/AU]; Victoria Street, Darlinghurst, NSW 2010 (AU). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> GECZY, Andrew, Francis [AU/AU]; 10/3 Yeo Street, Neutral Bay, NSW 2098 (AU). RUSSELL-JONES, Gregory, John [AU/AU]; 23 Greenfield Avenue, Middle Cove, NSW 2070 (AU). BELL, Stephen, John, Duncan [AU/US]; 4506 Wimbledon Way, Kalamazoo, MI 49009 (US). COOPER, David, Albert [AU/AU]; 7 Carrington Avenue, Bellevue Hill, NSW 2023 (AU).		<b>(74) Agent:</b> F.B. RICE & CO.; 28A Montague Street, Balmain, NSW 2041 (AU). <b>(81) Designated States:</b> AT, AU, BB, BG, BR, CA, CH, CS, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG). <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> IMMUNOSTIMULATION  <b>(57) Abstract</b>  The present invention provides novel compositions and methods for increasing immune responsiveness, in particular T-cell responsiveness in patients with an immunodeficiency, particularly in T-cell function. The present invention is particularly useful for increasing responsiveness of helper T-cells. The method of the present invention comprises administering to a patient a protein selected from the group consisting of TraT, OmpA, OmpF and parts thereof and a pharmaceutically acceptable carrier. Preferably, at least one other antigen is administered. The present invention should be particularly useful in the treatment of HIV positive patients.		

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ImmunostimulationField of the Invention

The present invention relates to the use of costimulator inducers to augment or to boost the immune response in patients with deficiencies in T-cell function, in particular helper T-cell function, and to novel compositions useful in increasing responsiveness of T-cells, in particular helper T-cells. More specifically, the invention relates to the use of the E. coli outer-membrane proteins OmpA, OmpF or TraT to augment the immune response to antigens in immunocompromised individuals and to compositions including these proteins.

Background of the Invention

During the development of an immune response, a certain type of T-cell, known as the helper T-cell, which bears the CD4 phenotype, is required to assist the B-cell to differentiate into a plasma cell which in turn secretes soluble antibody. Before undergoing activation and proliferation, helper T-cells, with the aid of the T-cell receptor and other accessory molecules, must first recognise antigens on the surface of antigen-presenting cells (APC) such as macrophages, dendritic cells or B-cells. More recent data are consistent with the hypothesis that T-cells require two signals for their activation. One signal is delivered as a result of the binding of a peptide to a Class II Major histocompatibility complex (MHC) molecule on the APC, and the subsequent interaction of this MHC-peptide complex with the T-cell receptor. Although a necessary condition, T-cell receptor occupancy by the MHC-peptide and its associated biochemical consequences are not sufficient to induce T-cell activation. For most cells, a second signal or co-stimulator molecule must be provided by the APC (Lafferty, Prowse and Simeonovic, 1983; Mueller, Jenkins and Schwartz, 1989). Bacterial products, such as LPS and

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BCG, which have been shown to elicit co-stimulator activity from APC are known as costimulatory inducers (Janeway, 1990). It is suggested that costimulation inducers will augment or improve the immune response of individuals suffering from Immunodeficiency syndromes which are associated, at least in part, by a lack of helper T-cells.

Acquired Immunodeficiency Syndrome (AIDS) is a debilitating disease of man characterized by high morbidity and mortality of infected individuals. The disease, which is characterized by an initial infection with a lentivirus, the Human immunodeficiency virus (HIV), is diagnosable in its early stages by the presence of antibodies in serum against the HIV and/or the presence of the virus in the serum of asymptomatic individuals. Almost without exception these asymptomatic individuals go on to develop full blown AIDS with its many associated complications, which ultimately leads to death of the infected individuals.

During the course of the disease, HIV positive individuals show a progressive depletion of their helper T-cell population [CD4-positive cells ( $CD4^+$ )] with an increase in the numbers of  $CD8^+$  cells. Accompanying the loss of  $CD4^+$  cells, infected individuals show a progressive loss of their ability to mount a protective immune response to HIV, or to a number of opportunistic pathogens which may invade the infected individuals. This chronic depletion of helper ( $CD4^+$ ) T-cells, and the resultant impairment of cell-mediated immunity, correlates closely with disease progression towards AIDS (Fahey et al, 1990; Lange et al, 1989).

In an attempt to halt the spread of AIDS amongst at-risk individuals, and to develop a cure for the treatment of infected individuals, many research groups have concentrated their efforts on the development of a

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vaccine against the HIV. Thus, since the initial isolation of the HIV, some 10 years ago, many sophisticated virological and biotechnological approaches have been used in the design and production of a multitude of candidate vaccines (Hu et al, 1987; Kennedy et al, 1987). Almost without exception the various vaccine candidates have failed dismally in clinical trials. The failure of the many vaccine candidates and the inexorable progression of the disease in infected patients towards full blown AIDS, has led many to the pessimistic view that the development of an effective AIDS vaccine is almost impossible. This view is not, however, shared by Desrosiers and co-workers (1989) who have recently reported promising results vaccinating rhesus monkeys with inactivated HIV preparations.

One of the major problems in the development of an effective vaccine to combat already established HIV infections is that the HIV itself invades and inactivates the CD4<sup>+</sup> helper T-cells, which are the very cells which must be stimulated for an individual to mount a protective immune response. During the course of a normal immune response, activated CD4<sup>+</sup> helper cells produce cytokines such as Interleukin-2 (IL-2) which are known to drive the clonal proliferation of primed T-cells, which can ultimately lead to the elimination of virally infected cells. It has been shown, however, that the addition of exogenous IL-2 to the peripheral blood mononuclear cells (PBMC) of HIV-seropositive individuals, can restore both antigen- and mitogen-driven blastogenesis in vitro (Bell et al., 1990). Similar stimulation of PBMC in vivo, leading to the production of CD4-derived IL-2, would help to maintain strong CD8-associated antiviral immunity. It would appear, therefore, that there is considerable merit in adopting therapies that lead to an increase in the level of CD4<sup>+</sup> T-lymphocytes, particularly during the

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asymptomatic stage of HIV-induced disease.

Nevertheless, IL-2 which has been recommended for approval by the FDA ( Stone R., Science, 255, 528, 1992) as an immunotherapeutic for the treatment of kidney cancer, has a number of disturbing side effects including...."circulatory problems that can be as severe as heart attacks and strokes".... There is clearly a need therefore for an effective immunostimulant which is devoid of the undesirable side effects attributed to IL-2.

One approach to improving the prognosis of HIV-infected individuals, would be to increase either the absolute number, or the responsiveness of helper T-cells in HIV-infected individuals and thereby improve the individual's capacity to mount an immune-attack on HIV-infected cells and to develop effective responses to opportunistic pathogens.

In international patent application No PCT/AU87/00107 it is disclosed that in complexes with an immunogen TraT, OmpA and OmpF act as potent immunoadjuvants in immunocompetent hosts. There is, however, no disclosure in this reference that TraT, OmpA or OmpF have any ability to increase the responsiveness of helper T-cells.

The present inventors have made the surprising finding that these proteins increase the responsiveness of helper T-cells from patients suffering a deficiency in helper T-cell function. Further, the present inventors have found a synergistic effect on helper T-cell responsiveness between these proteins and other antigens.

#### Summary of the Present Invention

Accordingly, in a first aspect the present invention consists in a composition comprising in admixture a protein selected from the group consisting of TraT, OmpA, OmpF and parts thereof, at least one other antigen and a pharmaceutically acceptable carrier.

In a second aspect the present invention consists in

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a composition comprising a protein selected from the group consisting of TraT, OmpA, OmpF and parts thereof, coupled to an antigen selected from the group consisting of HIV antigens, influenza virus antigens, diphtheria antigens, whooping cough antigens, measles antigens, tetanus antigens, Pneumocystis antigens, Candida antigens, Toxoplasmosis antigens, Cytomegalovirus antigens, hepatitis antigens, polio antigens, combinations thereof and individual subunit proteins, peptides or polysaccharides isolated from said antigens, and a pharmaceutically acceptable carrier.

In a third aspect the present invention consists in a method of increasing immune responsiveness in a patient with an immunodeficiency, the method comprising administering to the patient a composition comprising an effective amount of a protein selected from the group consisting of TraT, OmpA, OmpF and parts thereof and a pharmaceutically acceptable carrier.

In a fourth aspect the present invention consists in the use of a composition comprising an effective amount of a protein selected from the group consisting of TraT, OmpA, OmpF and parts thereof and a pharmaceutically acceptable carrier, diluent and/or excipient in the manufacture of a medicament for increasing immune responsiveness in a patient with a deficiency in immune function.

In a preferred embodiment of the present invention the responsiveness of T-cells is increased and the patient has a deficiency in T-cell function.

In a further preferred embodiment of the present invention the T-cells are helper T-cells and the patient has a deficiency in helper T-cell functions.

In a preferred embodiment of the present invention the pharmaceutically acceptable carrier is a hydrophobic depot carrier. Suitable depot carriers include alhydrogel, proteosomes and liposomes.

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In a further preferred embodiment of the present invention the at least one other antigen is selected from the group consisting of HIV antigens, influenza virus antigens, diphtheria antigens, whooping cough antigens, 5 measles antigens, tetanus antigens Pneumocystis antigens, Candida antigens, Toxoplasmosis antigens, Cytomegalovirus antigens, hepatitis antigens, polio antigens and combinations thereof and individual subunit proteins, peptides or polysaccharides isolated from said antigens.

10 It is presently preferred, however, that the at least one other antigen is a HIV antigen, diphtheria toxoid or tetanus toxoid, and most preferably a HIV antigen selected from gp41[8] peptide and V3 loop peptide.

In yet a further preferred embodiment of the present 15 invention the protein is TraT or a part thereof.

TraT, OmpF and OmpA are outer membrane proteins of Gram negative bacteria. The TraT protein is an outer membrane protein of certain strains of E.coli which is responsible for the resistance of these strains to killing 20 by serum. The OmpA and OmpF proteins also fall in the same class of proteins. These proteins may be obtained from other Gram negative bacteria such as E.coli or Salmonella species. It is, however, presently preferred that the proteins are obtained from strains of E.coli.

25 The studies presented in this invention have shown an ability to enhance the level and/or activity of helper T-cells in individuals with deficiencies in helper T-cell function which indicates that TraT, OmpF and OmpA of E. coli and parts thereof can function as costimulator 30 inducers separate from, but with similar function to, the costimulator inducers BCG and LPS, as described by Janeway (1990).

The present inventors have shown that the costimulator inducer activity of outer membrane proteins TraT and OmpA



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of E. coli can be used to enhance the stimulation of helper T-cells, derived from HIV-positive individuals, in the presence of antigen, and specifically, peptides derived from the viral proteins or recall antigens such as

5 Diphtheria toxoid (DT) and Tetanus toxoid (TT).

In contrast to BCG and LPS, TraT, OmpA and OmpF do not produce undesirable side-effects such as endotoxic shock and granuloma formation at the injection site.

TraT, OmpF and OmpA can, therefore, be used as  
10 inducers of costimulatory activity in antigen presenting cells and thereby stimulate helper T-cells in the induction of immune responses to, for instance, a number of HIV-derived antigens, and thereby overcome the CD4-positive T-cell non-responsiveness in HIV-infected individuals.

15 The clinical outcome of increased helper T-cell numbers is improved immune function which in turn will result in an increased capacity of an individual to combat opportunistic infections.

The use of these molecules would greatly improve the  
20 efficacy of candidate AIDS vaccines by stimulating the production of helper T-cells. Alternatively, when used in conjunction with other antigens to which an individual has previously developed memory T-cells, these molecules will enhance the overall level of immunity of the individual.

25 The ability of these molecules to restore helper T-cell function could also be exploited to enhance helper T-cell production in immunodeficiency conditions such as those which may arise following certain types of cancer, organ transplantation and various autoimmune conditions.

30 The compositions of the present invention are prepared by mixing, preferably homogeneously mixing, TraT, OmpA or OmpF or a part of TraT, OmpA or OmpF, which part stimulates an antigen presenting cell to provide a costimulator signal for helper T-cells, with a pharmaceutically acceptable  
35 carrier, diluent, and/or excipient using standard methods

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of pharmaceutical preparation.

Preferably the method additionally comprises using at least one other antigen in the preparation of the pharmaceutical composition. The antigen may be an antigen  
5 against which it is desirable to raise an immune response in the patient. For instance in AIDS patients HIV antigens may be used. Other antigens which might be used include influenza virus antigens, diphtheria antigens, whooping cough antigens, measles antigens Pneumocystis antigens,  
10 Candida antigens, Toxoplasmosis antigens, Cytomegalovirus antigens, combinations thereof and individual subunit proteins, peptides or polysaccharides isolated from said antigens.

The TraT, OmpA and OmpF proteins which can be used in  
15 accordance with the present invention may be purified from publicly available standard E. coli strains which produce these proteins.

One such strain of E. coli is ATCC 67331 which was deposited with the American Type Culture Collection of  
20 12301 Parklawn Drive, Rockville MD 20852, U.S.A. on 2 March 1987. Purification of TraT, OmpF and OmpA from E. coli is described in International Patent Application No. PCT/AU87/00107 (WO 87/06590).

Alternatively these proteins may be obtained from  
25 other bacterial strains which carry recombinant DNA molecules encoding these proteins, and purified by a method appropriate to the site of production of the recombinant TraT, OmpA or OmpF protein.

Where parts of these proteins, which stimulate an  
30 antigen presenting cell to provide a costimulatory activity for helper T-cells are to be used, the required parts can be identified and prepared as follows.

The intact molecule is employed to identify the receptor which binds the molecule on the antigen presenting  
35 cell. The intact molecule is then digested by standard

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protein digestion techniques and the parts generated are assayed for binding to the identified receptor. Those parts which can bind and stimulate production of costimulatory activity by the antigen presenting cell are  
5 suitable for use in the compositions and methods of the present invention.

As will be readily understood by persons skilled in the art homologues and analogues of TraT, OmpA and OmpF could be used in the present invention with similar  
10 effect. It is intended that the use of such homologues and analogues are encompassed within the scope of this application.

The antigens to be used in compositions and method of the present invention may be any antigen against which it  
15 is desirable to raise an immune response in an immunocompromised or immunosuppressed patient.

Examples include, for instance, antigens of the HIV such as gp41[8] peptide which may be of use to stimulate blastogenesis of HIV-specific lymphocytes in HIV-infected  
20 patients. Other antigens might include influenza virus antigens, diphtheria antigens, whooping cough antigens, measles antigens Pneumocystis antigens, Candida antigens, Toxoplasmosis antigens, Cytomegalovirus antigens, combinations thereof and individual subunit proteins,  
25 peptides or polysaccharides isolated from said antigens.

The compositions of the invention may be prepared using standard pharmaceutical techniques.

Where an antigen is to be used in the composition, it may be admixed with the costimulator inducer in the depot.  
30 Alternatively, the antigen and costimulator inducer may be complexed by chemical conjugation using chemical modification and/or linking groups where required. For proteinaceous antigens, the costimulator inducer and antigen could be provided as a fusion protein, by  
35 recombinant DNA techniques.

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In each case, it is to be understood that the process for joining the antigen to the costimulator inducer should not destroy the desired antigenicity of the antigen or the costimulator inducer activity of the TraT, OmpA, OmpF or  
5 part thereof.

The costimulator-inducer or costimulator-inducer and antigen can be formulated in a depot carrier. Where both components are to be included it is desirable to keep them together. A depot carrier is suitable to achieve this and  
10 the types of depot carrier which can be used include alhydrogel, proteosomes and liposomes. The compositions are prepared by standard techniques appropriate to the carrier being used.

Where the costimulator-inducer is to be used without  
15 antigen or where the costimulator-inducer is complexed or fused to the antigen, traditional carriers other than depot carriers can also be used.

The composition of the present invention is preferably administered parenterally to the patient by standard  
20 techniques of parenteral administration.

Typically 100ng-10mg of each costimulator inducer and antigen is used in each dose.

The precise dose and ratio of each costimulator inducer and antigen to be used will depend on: (i) the type  
25 and nature (e.g. immunogenicity) of the antigen; (ii) the genetic background of the subject; (iii) the immunological history of the subject; and (iv) the type of immune response (e.g. humoral, lymphocyte-mediated, macrophage-mediated or granulocyte-mediated) one is seeking  
30 to modify.

A skilled addressee will be able to determine the appropriate ratio of costimulator inducer to antigen by systematically varying the relative dose and proportions of costimulator to antigen until the desired immune response  
35 has been achieved.

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It is recognised that a number of factors will affect the determination of an appropriate dosage for a particular patient. Such factors include the age, weight, sex, general health and concurrent disease status of the  
5 patient. The determination of the appropriate dose level for the particular patient is performed by standard pharmaceutical techniques.

Patients for whom the use of the methods and compositions of the invention is envisaged are patients  
10 having a deficiency in helper T-cell function such as patients suffering from disease states including autoimmune diseases, some cancers and AIDS, and patients where an immunosuppressed state is artificially induced during treatment of a particular disease state or condition, for  
15 instance transplant patients and cancer patients undergoing chemotherapy or radiotherapy.

The method of the invention might be used to raise their helper T-cell levels in general or the inclusion of specific antigens can be desirable in order to raise helper  
20 T-cell levels in order to protect the patient from specific infections which could prove fatal in their immunocompromised or immunosuppressed state.

While it is understood that the primary focus of the present invention is the treatment of human patients the  
25 present invention is equally applicable for the treatment of non-human animals. Accordingly, as used herein the term "patient" is intended to cover both non-human and human animals.

In order that the nature of the present invention may  
30 be more clearly understood, preferred forms thereof will now be described with reference to the following examples.

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EXAMPLE 1.

(i) TraT augments the in vitro T-cell proliferative responses elicited by immunodominant HIV-derived synthetic peptides.

5 A. Selection of the HIV-derived peptide (gp41[8]).

The majority of the immunodominant sequences of the Human Immunodeficiency virus type-1 (HIV-1) are coded by hypervariable gene sequences and these sequences are interspersed with regions that are highly conserved  
10 amongst HIV-1 strains. Immunogenic viral proteins that show minimal strain-to-strain variation and that consistently elicit both humoral and cell-mediated immune responses may be useful components for inclusion in a subunit vaccine. In this connection, Bell and co-workers  
15 (Bell et al, 1992) have studied HIV-seronegative subjects and HIV-infected individuals classified as asymptomatic (AS), as AIDS-related complex (ARC) or as AIDS. In accordance with the clinical classification system of the CDC (Centers for Disease Control, 1986), AS HIV-infected  
20 individuals constituted CDC Group II/III; ARC patients were CDC Group IVA/IVC2, and AIDS were CDC Group IVCI/IV D. They initially determined which of three short synthetic peptides derived from the conserved sequences of the envelope gp 120 (amino acids 262-284), gp41 (aa 579-601),  
25 and core p17 (aa 106-125) regions of the HTLV-III<sub>B</sub> isolate, could elicit B-cell as well as T-cell responses in HIV infected individuals. Only the gp41-derived sequence was immunogenic at the B- and T-cell levels. The gp41 region was characterized further by using a series of  
30 overlapping synthetic peptides derived from a conserved region of the envelope gp41 (aa 572-613). The authors subsequently identified an immunodominant dodecamer (aa 593-604; termed gp41[8]) which consistently evoked both T-blastogenic and antibody responses in asymptomatic  
35 HIV-seropositive individuals to a lesser extent in ARC,

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but not in AIDS patients.

**B. The synthesis of HIV-1-derived peptides.**

The two peptides, gp41[8] and V3 loop derived from the gp120 region of HIV-1, were synthesized on an Applied Biosystems No. 430A peptide synthesizer following the manufacturers instructions. The peptides were purified by chromatography on G-25 Sephadex (Pharmacia) in 10% Acetic Acid, followed by Reverse Phase HPLC on a VYDAC C-18 column using a linear gradient of 5-60% acetonitrile in 0.1% TFA. The sequences of the peptides synthesised are as follows:

R-S-S-gp41[8]: To improve the solubility of this peptide, Arg-Ser-Ser was added to the amino terminal end of the gp41[8] sequence viz.,  
Arg-Ser-Ser-Leu-Gly-Ile-Trp-Gly-Cys-Ser-Gly-Lys-Leu-Ile-Cys.

**V3 loop peptide:**

Asn-Thr-Arg-Lys-Ser-Ile-Arg-Ile-Gln-Arg-Gly-Pro-Gly-Arg-Ala-Phe-Val-Thr-Ile-Gly-Lys-Ile-Gly-Asn.

20

**C. Assessment of human T-cell proliferative responses.**

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque (Pharmacia) gradient centrifugation, and 200,000 PBMC were cultured in 0.2 ml RPMI-160 medium (Cytosystems Pty. Ltd., containing 10% human AB serum, 50 mM each of penicillin and streptomycin, 2.5 mM glutamine and 2 mM HEPES buffer solution, pH 7.4) in 96-well round-bottom microtitre plates in the presence of gp41[8] or V3 loop synthetic peptides (2 $\mu$ M), rIL-2 (10u/ml), Diphtheria toxoid (DT; Commonwealth Serum Laboratories, Melbourne, Australia, 1570Lf units/ml; 4 and 40  $\mu$ g/ml), Tetanus toxoid (TT; Commonwealth Serum Laboratories, Melbourne, Australia, 100Lf units/ml; 5 and 50  $\mu$ g/ml); TraT and OmpA (purified as described by Croft et al. 1991; 40  $\mu$ g/ml). After 6 days of culture at 37°C

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(with or without antigen), each culture was pulsed overnight with 50 $\mu$ l of  $^3$ H-thymidine (20 $\mu$ Ci/ml; Amersham, U.K.), harvested on glass-fibre filter papers and counted in a liquid scintillation spectrometer (Beckman, U.S.A.).

- 5 Results were expressed as Stimulation Indices (S.I.) which were calculated as follows:

$$\text{S.I.} = \frac{\text{mean counts per minute (c.p.m.) with antigen} - \text{mean c.p.m. without antigen}}{\text{mean c.p.m. without antigen}}$$

- 10 D. Definition of TraT- and IL-2-mediated effects on gp41[8]-, V3 peptide-, Diphtheria toxoid (DT)-, or Tetanus toxoid (TT)- specific lymphoproliferation.

The effects of TraT and IL-2 on proliferative responses (expressed as Stimulation Indices) to the various

- 15 HIV-derived and recall (DT and TT) antigens have been defined by using a modified version of a documented formula (Denz et al., 1985):

An additive effect was defined as:

$$\frac{(AB)}{(A) + (B)} = 1$$

A Synergistic effect was defined as:

$$\frac{(AB)}{(A) + (B)} > 1$$

and an Inhibitory effect was defined as:

$$\frac{(AB)}{(A) + (B)} < 1$$

where (A) and (B) signify the proliferative responses to the individual agents (e.g., gp41 [8] and TraT), respectively, and (AB) is the lymphoproliferative response seen when both agents are combined and added to the same cultures.



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### Results

The addition of TraT to cultures of PBMC from eleven asymptomatic (AS) individuals, thirteen patients with AIDS-related complex (ARC) and one AIDS-lymphoma patient, augmented the T-cell proliferative response to the gp41[8] peptide to levels which were higher than that achieved by the addition of IL-2 to cultures containing the gp41[8] peptide (Table 1). In every case, with the exception of patient #110, a synergistic effect was seen in cultures that had been stimulated with a mixture of TraT and gp41[8] and this effect was maximal when 40 µg of TraT was used in the cell cultures (Table 2). By contrast, a synergistic effect was observed in only three from twenty-five cases, when IL-2 was combined with gp41[8] (Table 1). The effect of another outer-membrane protein, OmpA, was also tested on PBMC cultures of one asymptomatic, and one symptomatic individual, and in both cases a synergistic effect was seen when OmpA was co-cultured with gp41(8) (Table 1).

In view of the impressive results obtained with gp41[8] in the presence of TraT, it was important to determine whether TraT would augment the T-cell response to another HIV-derived peptide. The V3 loop peptide was considered a suitable candidate, as this peptide (La Rosa et al., Science 249: 932, 1990), the principal neutralising determinant of HIV-1, is currently in clinical trials (Scrip, No. 1703, 26, 1992). The results in Table 3 show that in all eight cases tested TraT augmented the proliferative responses to the V3 peptide. However, an inhibitory effect was observed when PBMC from the eight individuals were cultured in the presence of IL-2 and V3 peptide (Table 3). In summary, TraT was far more effective than IL-2, a lymphokine which has been trialled as an Immunotherapeutic (Rosenberg, Lotze and Mul , 1988), in augmenting the T-cell responses to the HIV-derived

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peptides gp41[8] and the V3 loop.

- (ii) TraT augments the in vitro T-cell proliferative responses to recall antigens such as Diphtheria toxoid (DT) and Tetanus toxoid (TT).

It is well established that lymphoproliferative responses to recall antigens are impaired even during the asymptomatic disease period when CD4-positive T-cell numbers are often only slightly reduced (Lane et al., 1985). Since TraT has been shown to augment HIV-specific lymphoproliferation in both symptomatic and asymptomatic individuals, it was reasonable to expect that it would have a similar effect in enhancing the response to recall antigens. The ability of TraT to enhance the response to recall antigens will be of clinical importance in boosting immunity and thereby enabling immunocompromised individuals to combat opportunistic infections. In all six patients studied (#123 to #128), TraT significantly augmented DT - and T T- specific proliferative responses (Table 4). This finding would make TraT an attractive immunomodulator molecule for restoring defective T-cell responses, not only to HIV-derived antigens, but also to a range of recall antigens. From a clinical viewpoint, a molecule with co-stimulator inducer-like properties such as TraT would be an attractive immunotherapeutic for boosting general immunity in immunocompromised individuals.

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EXAMPLE 2

Flow cytometric analysis of T-cell subset distribution indicates that TraT preferentially stimulates CD4-positive helper T-cells.

- 5 The T-cell subset distribution of Peripheral blood mononuclear cells (PBMC), that had been stimulated with TraT, Interleukin-2 (IL-2) or with gp41[8], after a 6-day incubation, were analysed using immunofluorescence and flow cytometry.
- 10 The phenotypes of the T-cells in proliferating cultures of PBMC were compared with those from unstimulated cell cultures.

T-cell phenotype analysis.

- Two-millilitre volumes of both stimulated and unstimulated
- 15 PBMC ( $5 \times 10^6$  cells/ml) were cultured for 6 days under the same conditions as described in Example 1. After 6 days of culture, pelleted cells were resuspended, and the cell suspensions layered onto 1 ml Ficoll-Hypaque gradients (Pharmacia), and centrifuged at 800g for 10
- 20 min. Viable (as judged by Trypan blue exclusion) lymphoblastoid cells were collected from the interface and washed in Hank's Balanced Salt Solution (HBSS; Cytosystems, Pty. Ltd.) pH 7.4. Viable cells that had been isolated from unsorted cultures of PBMC were
- 25 phenotyped using dual combinations of fluorescent monoclonal antibodies (i.e., CD 3/4 and CD 3/8; Coulter Electronics Inc., Hialeah FL, U.S.A.). Two-thousand viable cells from each PBMC culture were assayed for surface-bound fluorescence. Using both the viable PBMC
- 30 count (i.e., after 6 days of culture with and without stimulation), and the relative percentages of the

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respective T-cell subsets gated in each bitmap of 2,000 cells, the absolute numbers of T-cells from the CD4-positive and CD8-positive T-cell subsets were calculated. The formula used for the calculation of  
5 subset numbers was: T-cell subset % (per 2,000 "gated" cells) x viable cell number (as determined by Trypan blue exclusion). Bitmaps were continually adjusted to encircle the majority of either stimulated or unstimulated lymphocytes. After 6 days of culture of purified PBMC,  $\geq$   
10 90% of viable cells were consistently found to be CD3-positive, indicating that they were predominantly T-cells.

There was a 23 to 48% increase in absolute CD4-positive T-cell numbers (based on absolute cell numbers in the  
15 unstimulated cultures) when PBMC were cultured in the presence of TraT, gp41[8]), or IL-2. However, when TraT was combined with gp41[8], CD4-positive T-cells increased by 63% (#123) and 96% (#124) respectively (Table 5). By contrast, there was a somewhat lower (0 to 42%) increase  
20 in CD8-positive T-cell numbers when PBMC were incubated in the presence of any of the three stimulants, and this increased to 10% (#123) and 79% (#124) when PBMC were exposed to a mixture of TraT and gp41[8] in a 6-day culture. Another notable feature of these results is that  
25 after a 6-day incubation with the various stimulants, there was a significant increase in the CD4/CD8 ratio compared with the CD4/CD8 ratio measured immediately after isolation of the PBMC.

The synergistic effect between TraT and gp41[8] was even  
30 more pronounced when these stimulants were tested on the PBMC from patients #125 and #126 (Table 6). In these two ARC patients, the percentage increases in CD4 cell numbers were: TraT, 58% (#125), 36% (#126); gp41[8], 0% (#125), 18% (#126); IL-2, 378% (#125), 216% (#126). However,  
35 there was a dramatic increase in CD4 cell numbers when

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TraT was combined with gp41[8]: 737% (#125) and 1,763% (#126) respectively. The percentage increases in CD4 were significantly higher than the corresponding increases for the CD8-positive population, i.e., 74% (#125) and 185% (#126) (Table 5). The preferential increase in CD4-positive helper T-cells in cultures incubated with TraT, or with a combination of gp41[8] and TraT, suggests that TraT will boost helper T-cell numbers in vivo and thereby enable HIV-infected individuals to combat opportunistic infections. Further improvement in HIV-positive individuals would be obtained by combining TraT with anti-retroviral agents such as zidovudine.

#### Industrial Application

The current invention is applicable to the preparation of vaccines designed to combat immunodeficiency disorders such as AIDS and to the treatment of patients suffering a deficiency in helper T-cell function in general.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

**TABLE 1** The augmentation by Trat of gp41[8]-specific T-cell proliferative responses  
Treatments (Stimulation Indices)

Patient No.	Diagnosis	Trat	gp-41[8]	IL-2	gp41[8] + IL-2	gp41[8] + Trat	OmpA	gp41[8]+OmpA
100	AIDS-lymphoma	8.0	0.6	1.2	1.0(I)*	21.5(S)	nt	nt
101	Mild ARC	0.6	2.2	0.4	10.9(S)#	17.8(S)	nt	nt
102	Mild ARC	0.9	2.5	2.0	1.6(I)	7.0(S)	nt	nt
103	Mild ARC	3.3	3.3	2.6	1.1(I)	21.2(S)	nt	nt
104	AS	8.5	52.1	26.2	nt	163.0(S)	nt	nt
105	AS	26.6	66.7	27.4	nt	252.0(S)	nt	nt
106	AS	13.4	12.8	11.7	nt	50.5(S)	nt	nt
107	AS	13.1	15.8	41.1	nt	48.7(S)	nt	nt
108	AS	3.0	2.0	4.9	nt	15.9(S)	nt	nt
109	AS	3.5	3.6	6.8	nt	28.3(S)	nt	nt
110	Mild ARC	7.2	5.6	2.9	nt	13.1(A)**	nt	nt
111	Mild ARC	8.0	10.5	6.3	14.0(I)	21.7(S)	nt	nt
112	AS	9.0	5.9	8.5	16.3(S)	39.7(S)	nt	nt
113	ARC	29.0	7.9	12.0	18.1(I)	48.0(S)	16.0	29.3(S)
114	AS	56.4	19.9	22.1	36.0(I)	99.3(S)	17.9	42.8(S)
115	ARC	6.9	12.2	29.1	44.1(S)	33.7(S)	nt	nt
116	ARC	8.0	9.0	23.5	34.8(A)	23.4(S)	nt	nt
117	ARC	8.0	13.1	28.5	43.8(A)	32.8(S)	nt	nt
118	AS	12.0	7.0	13.5	22.0(A)	34.5(S)	nt	nt
119	AS	9.9	3.5	8.3	11.4(A)	21.9(S)	nt	nt
120	Mild ARC	42.8	32.5	67.7	22.0(I)	109.7(S)	nt	nt
123	ARG- Kaposi's Sarcoma	10.4	6.3	8.5	13.8(I)	50.0(S)	nt	nt
124	AS	5.7	6.2	6.1	12.4(A)	38.2(S)	nt	nt
125	ARC	7.2	4.3	4.4	7.4(I)	24.1(S)	nt	nt
126	ARC	12.2	6.9	8.7	12.4(I)	32.9(S)	nt	nt

\*I = Inhibitory effect; /S = Synergistic effect; \*\*A = Additive effect. nt = not tested  
For an explanation of these terms see Example 1

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TABLE 2

Dose-Response effect of TraT  
on gp41[8] - specific T-cell proliferation

	<u>Patient No. and Diagnosis</u>			
Treatments	111	112	113	114
	Mild			
	ARC	AS	AS	AS
<hr/>				
	Stimulation Indices			
<hr/>				
TraT - 10μg	3.0	4.3	7.8	16.5
TraT - 20μg	5.2	7.8	18.1	35.2
TraT - 40μg	8.0	9.0	29.0	56.4
TraT - 60μg	9.0	7.7	23.2	42.5
TraT - 80μg	5.4	6.8	14.7	25.7
gp41[8]	10.5	5.9	7.9	19.9
TraT (10μg) + gp41[8]	13.3	25.9	20.2	38.6
TraT (20μg) + gp41[8]	17.2	25.3	35.5	75.8
TRAT (40μg) + gp41[8]	21.7	39.7	48.0	99.3
TRAT (60μg) + gp41[8]	24.8	18.8	30.8	70.0
TRAT (80μg) + gp41[8]	19.3	17.8	24.5	48.2

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TABLE 3

The augmentation by TrAT of V3 loop peptide-specific T-cell proliferative responses

## Treatments (Stimulation Indices)

Patient No.	Diagnosis	TrAT	V3	IL-2	V3 + IL2	V3 + TrAT
125	ARC	7.2	3.8	4.4	6.9(I)	18.7(S)
126	AS	12.2	5.6	8.7	10.6(I)	26.0(S)
127	AS	12.1	6.3	8.5	10.0(I)*	30.9(S)
128	Mild ARC	4.6	6.7	8.1	11.8(I)	36.9(S)
129	AS	3.7	2.7	4.1	3.7(I)	21.0(S)
130	AS	5.5	4.5	6.7	5.7(I)	30.9(S)
131	ARC-Kaposi's					
	Sarcoma	5.4	4.4	6.9	5.7(I)	30.9(S)
132	ARC	4.1	5.0	5.7	6.3(I)	22.6(S)

\* I = Inhibitory effect; S = Synergistic effect



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TABLE 4

The augmentation by TraT of T-cell proliferative responses to  
Diphtheria toxoid (DT) and Tetanus toxoid (TT)

Treatments	<u>Patient No and Diagnosis</u>					
	123 Kaposi's Sarcoma	124 AS	125 ARC**	126 ARC	127 AS	128 Mild ARC
	(Stimulation Indices)					
TraT(40µg)	10.4	5.7	7.2	12.2	12.1	11.2
DT(40µg)	6.5	5.3	3.3	5.1	6.2	6.1
DT(4µg)	4.0	2.7	1.4	1.41	3.31	3.9
TT(50µg)	4.5	5.4	2.8	3.7	5.5	3.0
TT(5µg)	3.0	2.8	1.6	2.7	3.1	2.7
TraT+DT(40µg)	37.6(S)*	27.0(S)	20.9(S)	24.0(S)	30.9(S)	30.2(S)
TraT+DT(4µg)	21.6(S)	18.7(S)	11.5(S)	21.6(S)	23.8(S)	19.0(S)
TraT+TT (50µg)	34.0(S)	27.5(S)	16.0(S)	29.5(S)	26.5(S)	21.1(S)
TraT+TT(5µg)	21.3(S)	13.4(S)	11.2(S)	19.5(S)	21.1(S)	19.6(S)

\* Synergistic effect

AS = Asymptomatic; \*\* ARC = AIDS-related complex

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TABLE 5

Flow cytometric analysis of T-cell subset distribution of  
proliferating and unstimulated cells after a 6-day incubation

Patient No. and Diagnosis

#123 - Kaposi's Sarcoma*				#124 - Mild ARC*		
Absolute T-cell numbers				Absolute T-cell numbers		
Treatment	CD4	CD8	<u>CD4</u>	CD4	CD8	<u>CD4</u>
NIL	3.04x10 <sup>6</sup>	2.7x10 <sup>6</sup>	1.1	2.5x10 <sup>6</sup>	2.4x10 <sup>6</sup>	1.0
TraT	3.7x10 <sup>6</sup>	2.28x10 <sup>6</sup>	1.6	3.0x10 <sup>6</sup>	2.5x10 <sup>6</sup>	1.2
gp41[8]	3.8x10 <sup>6</sup>	2.40x10 <sup>6</sup>	1.6	3.7x10 <sup>6</sup>	2.9x10 <sup>6</sup>	1.3
IL-2	3.9x10 <sup>6</sup>	2.5x10 <sup>6</sup>	1.6	3.6x10 <sup>6</sup>	3.4x10 <sup>6</sup>	1.1
[8]+TraT	4.9x10 <sup>6</sup>	2.9x10 <sup>6</sup>	1.7	4.9x10 <sup>6</sup>	4.3x10 <sup>6</sup>	1.1
[8]+IL-2	4.1x10 <sup>6</sup>	2.4x10 <sup>6</sup>	1.7	3.6x10 <sup>6</sup>	3.8x10 <sup>6</sup>	0.9

\* CD4/CD8 at day 0 was #123=0.23; #124=0.52

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TABLE 6

Flow cytometric analysis of T-cell subset distribution  
of proliferating and unstimulated cells after  
a 6-day incubation

Patient No. and Diagnosis

#125 - ARC

#126 - ARC

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Absolute T-cell numbers

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Absolute T-cell numbers

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Treatment	CD4	CD8	$\frac{CD4}{CD8}$	CD4	CD8	$\frac{CD4}{CD8}$
<hr/>						
NIL	92,000	880,000	0.10	44,000	780,000	0.06
TraT	145,000	1,150,000	0.13	60,000	1,500,000	0.04
gp41[8]	54,000	980,000	0.06	52,000	550,000	0.09
IL-2	440,000	1,160,000	0.38	139,000	1,130,000	0.12
[8]+TraT	770,000	1,530,000	0.50	820,000	2,220,000	0.37
[8]+IL-2	29,000	1,280,000	0.03	68,000	1,310,000	0.05

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\* CD4/CD8 at day 0 was #125 = 0.125; #126 = 0.09

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## CLAIMS:-

1. A composition comprising in admixture a protein selected from the group consisting of TraT, OmpA, OmpF and parts thereof, at least one other antigen and a  
5 pharmaceutically acceptable carrier.
2. A composition as claimed in claim 1 in which the pharmaceutically acceptable carrier is a hydrophobic depot carrier.
3. A composition as claimed in claim 1 or claim 2 in  
10 which the at least one other antigen is selected from the group consisting of HIV antigens, influenza virus antigens, diphtheria antigens, whooping cough antigens, measles antigens, tetanus antigens Pneumocystis antigens, Candida antigens, Toxoplasmosis antigens, Cytomegalovirus  
15 antigens, hepatitis antigens, polio antigens, combinations thereof and individual subunit proteins peptides or polysaccharides isolated from said antigen.
4. A composition as claimed in claim 3 in which the at least one other antigen is a HIV antigen selected from  
20 gp41[8] peptide and V3 loop peptide.
5. A composition as claimed in any one of claims 1 to 4 in which the protein is derived from E.coli.
6. A composition as claimed in any one of claims 1 to 5 in which the protein is TraT or a part thereof.
- 25 7. A composition comprising a protein selected from the group consisting of TraT, OmpA, OmpF and parts thereof, coupled to an antigen selected from the group consisting of HIV antigens, influenza virus antigens, diphtheria antigens, whooping cough antigens, measles antigens,  
30 tetanus antigens Pneumocystis antigens, Candida antigens, Toxoplasmosis antigens, Cytomegalovirus antigens, hepatitis antigens, polio antigens, combinations thereof and individual subunit proteins, peptides or  
polysaccharides isolated from said antigen, and a  
35 pharmaceutically acceptable carrier.

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8. A composition as claimed in claim 7 in which the antigen is selected from the group consisting of HIV antigens, diphtheria toxoid and tetanus toxoid.
9. A composition as claimed in claim 8 in which the HIV antigen is gp41[8] peptide or V3 loop peptide.
10. A composition as claimed in any one of claims 7 to 9 in which the protein is TraT or a part thereof.
11. A method of increasing immune responsiveness in a patient with an immunodeficiency, the method comprising administering to the patient a composition comprising an effective amount of a protein selected from the group consisting of TraT, OmpA, OmpF and parts thereof and a pharmaceutically acceptable carrier.
12. A method as claimed in claim 11 in which the responsiveness of T-cells is increased in a patient with a deficiency in T-cell function.
13. A method as claimed in claim 12 in which the T-cells are helper T-cells and the patient has a deficiency in helper T-cell function.
14. A method as claimed in any one of claims 11 to 13 in which the composition further includes an effective amount of at least one other antigen.
15. A method as claimed in any one of claims 11 to 14 in which the pharmaceutically acceptable carrier is a hydrophobic depot carrier.
16. A method as claimed in any one of claims 11 to 15 in which the protein is TraT or a part thereof.
17. A method as claimed in any one of claims 14 to 16 in which the at least one other antigen is selected from the group consisting of HIV antigens, influenza virus antigens, diphtheria antigens, whooping cough antigens, measles antigens, tetanus antigens Pneumocystis antigens, Candida antigens, Toxoplasmosis antigens, Cytomegalovirus antigens, hepatitis antigens, polio antigens, combinations thereof, and individual subunit proteins, peptides or



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polysaccharides isolated from said antigen.

18. A method as claimed in any one of claims 13 to 17 in which the antigen is a HIV antigen selected from gp41[8] peptide and V3 loop a peptide.

5 19. A method as claimed in any one of claims 13 to 18 in which the protein and the at least one other antigen are in admixture.

20. A method as claimed in any one of claims 11 to 19 in which the patient is HIV positive.

10 21. A method as claimed in any one of claims 11 to 20 in which the protein is derived from E.coli.

22. The use of a composition comprising an effective amount of a protein selected from the group consisting of TraT, OmpA, OmpF and parts thereof and a pharmaceutically  
15 acceptable carrier in the manufacture of a medicament for increasing immune responsiveness in a patient with a deficiency in immune function.

23. The use as claimed in claim 22 in which the medicament is for increasing the responsiveness of T-cells  
20 in a patient with a deficiency in T-cell function.

24. The use as claimed in claim 23 in which the T-cells are helper T-cells and the patient has a deficiency in helper T-cell function.

25. The use as claimed in any one of claims 22 to 24 in  
25 which the composition further includes an effective amount of at least one other antigen.

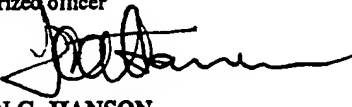
26. The use as claimed in any one of claims 22 to 25 in which the pharmaceutically acceptable carrier is a hydrophobic depot carrier.

30 27. The use as claimed in any one of claims 22 to 26 in which the protein is TraT or a part thereof.

28. The use as claimed in any one of claims 25 to 27 in which the at least one other antigen is selected from the group consisting of HIV antigens, influenza virus  
35 antigens, diphtheria antigens, whooping cough antigens,

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- tetanus antigens, measles antigens, Pneumocystis antigens, Candida antigens, Toxoplasmosis antigens, Cytomegalovirus antigens, hepatitis antigens, polio antigens, combinations thereof and individual subunit proteins, peptides or
- 5 polysaccharides isolated from said antigen.
29. The use as claimed in any one of claims 25 to 28 in which the antigen is a HIV antigen selected from gp41[8] peptide and V3 loop peptide.
30. The use as claimed in any one of claims 25 to 29 in
- 10 which the protein and the at least one other antigen are in admixture.
31. The use as claimed in any one of claims 22 to 30 in which the patient is HIV positive.
32. The use as claimed in any one of claims 22 to 31 in
- 15 which the protein is derived from E. coli.

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> Int. Cl. <sup>5</sup> A61K 39/108, 39/12, 39/39, C07K 13/00, 7/08, 7/10  According to International Patent Classification (IPC) or to both national classification and IPC				
<b>B. FIELDS SEARCHED</b>  Minimum documentation searched (classification system followed by classification symbols) Int. cl. <sup>5</sup> A61K 39/108, 39/12, 39/39, C07K 13/00, 7/08, 7/10  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU : IPC as above  Electronic data base consulted during the international search (name of data base, and where practicable, search terms used) DERWENT DATABASES : WPAT : K/W CHEM.ABS :K/W (K/W : E.COLI, OUTER MEMBRANE PROTEIN)				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>				
<b>Category*</b>	<b>Citation of document, with indication, where appropriate, of the relevant passages</b>	<b>Relevant to Claim No.</b>		
Y	EP 0109688 (The Wellcome Foundation Limited) 30 May 1984 (30.05.84) See abstract and claims	1,7		
Y	Derwent Abstract Accession No. 86-176377/27 Class B04,US,A, 6777068 (US Secretary of the Army) 1 April 1986 (01.04.86)	1,3,7		
Y	EP 0182401 (DESTAAT DER NEDERLANDEN VERTEGEN WOORDIGD DOOR DE MINISTER VAN WELZIJN, VOLKSGEZONDHEID EN CULTUR) 28 May 1986 (28.05.86). See abstract and claims	1,3,7		
<div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> Further documents are listed in the continuation of Box C.         </div> <div> <input checked="" type="checkbox"/> See patent family annex.         </div> </div>				
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="width: 50%; vertical-align: top;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> </td> </tr> </table>			<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>
<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p>			
Date of the actual completion of the international search 17 NOVEMBER 1992 (17.11.92)		Date of mailing of the international search report 24 NOV 1992 (24.11.92)		
Name and mailing address of the ISA/AU  AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA  Facsimile No. 06 2853929		Authorized officer   JOHN G. HANSON  Telephone No. (06) 2832262		

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
A	EP 0145359 (The Wellcome Foundation Limited) 19 June 1985 (19.06.85)	1,3,7
A	AU 42288/89 (PRAXIS BIOLOGICS, INC) 22 March 1990 (22.03.90)	1,3,7
A	US 4459286 (Hilleman et al.) 10 July 1984 (10.07.84)	1,3,7

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
EP	109688	US	4753796	EP	109688		
EP	182401	CA	1251396	EP	182401	JP	61171432
		GB	8316951				
EP	145359	DE	3483957	GB	8330968	US	4740589
AU	42288	EP	432220	WO	9002557	US	5098997
US	4459286	AU	23799/84	EP	117783	US	4459286
END OF ANNEX							

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